REMARKS

The foregoing amendment and remarks which follow are responsive to the initial, non-final Office Action mailed June 12, 2002 in relation to the above-identified patent application. In that Office Action, Claim 11 was objected to under 37 C.F.R. 1.75(c) as being in improper form because a multiple dependent claim cannot refer to more than one set of claims. It further was objected to based upon the presence of several inappropriate paragraph breaks. Claims 1-12 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and singly claim the subject matter which the applicant regards as the invention. In this regard, the Examiner considered the phrase "protecting brain cells" in Claim 1 as indefinite. Claims 11, 12, 14 and 15 were further deemed unclear or were otherwise improperly drafted.

With respect to the prior art, Claims 1, 2, and 5-10 were rejected under 35 U.S.C. § 102(b) as being anticipated by Akimoto et al., (i.e., United States Patent Number 5,889,046); Claims 1-7, 9 and 10 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Murakami et al. (i.e., United States Patent Number 5,151,450); and Claims 1, 3-7, 9, and 10 were rejected under 35 U.S.C. § 102(b) as being anticipated by the Derwent English abstract of Japanese Patent Number 05178793 A. Moreover, Claims 1, 3, 4, 6, 7, and 11-15 were rejected under 35 U.S.C. § 102(b) as

anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Akimoto et al.; Claims 1, 2, 6, and 7, were rejected under 35 U.S.C. § 103(a) as being unpatentable over Murakami et al. and Claims 1, 2, 6, 7; and 11-15 were rejected under 35 U.S.C. § 103(a) as being unpatentable over JP 05178793 A.

For the reasons set forth below, the claims of the present application are now believed to be in condition for allowance and Applicant requests reconsideration pursuant to 37 C.F.R. § 1.14 in light of the amendments made herein.

I. BACKGROUND OF THE INVENTION

By the present amendment, Applicant has more clearly claimed the subject matter which he regards as the invention, as well as to distinguish the same from the cited prior art. In this regard, the present invention is directed to compositions and methods for modulating certain biochemical reactions in the neuronal cells of mammals which protect such cells against damage caused by excitatory amino acids and oxidative stresses. Specifically, the invention comprises one or more extracts from Asiasari Radix that may be administered to a mammal, either by injection, topical application, oral administration, etc., in a therapeutically effective amount, which preferably ranges between 0.1 mg to 500 mg. Such extract is preferably derived via extraction with a lower alcohol and inorganic solvent. The extracts may further be

fractionated utilizing techniques well-known in the art to obtain more highly refined versions thereof.

In use, the Asiasari Radix extracts are administered at appropriate levels sufficient to effect at least one of three biochemical reactions related to the neuronal cells of a mammal. Specifically, such extracts are administered in therapeutically effective amounts to inhibit AMPA-induced depolarization, enhance tyrosine phosphorylation of hippocampal proteins (and in particular insulin receptors in the hippocampus), and/or inhibit hippocampal cholinesterase activity.

II. SUMMARY OF THE CITED PRIOR ART

The aforementioned references, Akimoto et al., Murakami et al., and JP05178793 A, have each been relied upon individually to maintain a rejection of the claims under 35 U.S.C. § 102(b) or, alternatively, 35 U.S.C. § 103(a). Each is discussed below.

A. United States Patent Number 5,889,046 to Akimoto et al.

The Akimoto et al. reference is directed to the use of dioxabicyclo [3.3.0] octane derivatives for preventing or alleviating cerebral apoplexy (i.e., stroke). Column 13, lines 2-20; Abstract. To achieve that end, such compounds are used in treating hypertension, both essential or secondary. The prevention or alleviation of cerebral apoplexy extends merely as a treatment

of a medical symptom caused by hypertension. Column 6, lines 21-35; Column 7, lines 10-31; and Column 8, lines 44-49. As such, it should be recognized that such reference is limited exclusively to a single class of compositions directed exclusively to the treatment of underlying hypertension and that such reference provides no teaching or suggestion whatsoever of a composition comprised of an extract having multiple therapeutically effective agents, much less the use thereof, for modulating specified neural cell biochemical reactions that impart a protective effect to brain cells.

B. United States Patent Number 5,151,450 to Murakami et al.

Murakami et al. is directed to 4,5-dihydroxy-2,6,6-trimethyl-2-cyclohepten-1-1 and the use thereof as an anti-ulcer agent. Column 1, lines 6-20; Column 8, lines 5-15; and Abstract. In this regard, such reference is drawn exclusively to such compound as the active ingredient. Id. As correctly pointed out in the Office Action, there is no discussion whatsoever of the use of such compound for use in imparting protection to brain cells against damages caused by excitatory amino acids and oxidated stresses, much less modulating neural cell biochemical reactions. Moreover, it should be noted that although referencing the fact that the active ingredient is derived from Asiasari Radix, the same does not reference any other active ingredients that, in combination to 4,5-

dihydroxy-2,6,6-trimethyl-2-cyclohepten-1-1, could further impart any sort of desired therapeutic effect.

C. JP05178793A

Japanese Patent Application Number 05178793A is directed to monoterpene derivatives that inhibit lipoxygenase activity that can be utilized as an anti-allergy and anti-inflammatory agent. Although the reference discloses deriving such compositions from an extract of Asarum sieboldii Miq., the same does not reference any type of combination of therapeutically effective compositions derived from such extract, let alone the use of such extract in memory enhancing and neuro protective applications.

III. APPLICANT'S CLAIMS AS AMENDED ARE NOVEL AND NON-OBVIOUS

A. NOVELTY

As is well-known, for anticipation to apply, all of the claimed elements must be found in exactly the same situation and united in the same way to perform the identical function in a single unit of the prior art. See, e.g., Studiengeselosehaft Kohle m.b.H. vs Dart Industries, 220 USPQ 841, 842 (Fed. Cir. 1984). In this respect, prior art reference cannot anticipate in terms of 35 U.S.C. § 102 unless every element of the claimed invention is

identically shown in a single reference. In re Bond, 15 USPQ 2d 1566, 1567 (Fed. Cir. 1999).

In light of the amendments made herein, none of the aforementioned references meet the standard. Akimoto et al., is directed to the use of a single compound, and does not utilize an extract of Asiasari Radix having multiple active ingredients. Moreover, and as correctly cited in the Office Action, such reference provides no teaching whatsoever for the use of any type of extract of Asiasari Radix for protecting brain cells or otherwise modulating neural cell biochemical reactions relating to AMPA-induced depolarization, tyrosine phosphorylation and/or cholinesterase inhibition.

The same shortcomings also apply to both the Murakami et al., and JP05178793A references. As discussed above, each reference is drawn to specific chemical entities that, although possibly being derived from an extract of Asiasari Radix, do not take into account or otherwise rely upon any other therapeutically effective agent, as does Applicant's extract. Moreover, and as again correctly indicated in the Office Action, such references in no way teach or suggest the new methods set forth in Claims 16 through 52 that are directed to methods of protecting brain cells and modulating brain biochemistry.

B. OBVIOUSNESS

It is similarly well-established that an obviousness rejection cannot be maintained based upon suggested modifications of the prior art unless the prior art suggested the desirability of such modifications. In re Fritch, 23 USPQ 2d, 1780, 1785 (Fed Cir. 1992). In light of the amendments made herein, all of the aforementioned references fail to meet the standard. In this regard, none of the aforementioned references suggest an extract of Asiasari Radix which includes multiple therapeutically effective agents and further, fail to suggest any type of method of protecting brain cells or otherwise effecting specific biochemical processes via the administration of a therapeutical amount of extract derived from Asiasari Radix.

In summary, none of the aforementioned references suggest any motivation for, or desirability of the invention as now claimed. Additionally, there is no secondary evidence that one skilled in the art would modify the compositions and/or methods disclosed in any of the aforementioned references to derive Applicant's extracts and methods of utilizing the same. Accordingly, Applicant respectfully submit that the amended claims and new claims submitted herein are allowable over the cited prior art.

IV. APPLICANT HAS FURTHER AMENDED THE CLAIMS AND APPLICATION TO PLACE THE SAME IN CONDITION FOR ALLOWANCE

With respect to the remaining outstanding issues regarding 35 U.S.C. § 112, second paragraph, Applicant has hereby amended the claims to specifically address the Examiner's concerns. Specifically, claims 1, 11, 12, 14 and 15 have been amended to address the specific issues raised in the outstanding Office Action. As such, rejection of such claims has been overcome.

Still further, Applicant has amended the specification to provide further clarification of the prior art, as well as provide additional clarification related to Applicant's examples. Applicant respectfully submits that such amendments to the specification do not introduce new matter and have been made solely to place the application in better condition for allowance. To that end, Applicant has further submitted herewith a new Figure 13, which more clearly shows the degree of tyrosine phosphorylation of insulin receptors is certain fractions of the Asiasari Radix extract of the present invention in rat hippocampus.

V. CONCLUSION

For the foregoing reasons, Applicant respectfully submits that all outstanding matters have been addressed and that the amended and newly submitted claims herein are allowable over the cited prior art. Early notice to that effect is respectfully

requested. To the extent the Examiner has any questions, requires any additional information, or has any suggestions to resolve any outstanding issues that may exist, he is invited to contact Applicant's counsel at the number listed below.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

If any additional fee is required, please charge Deposit Account Number 19-4330.

Respectfully submitted,

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"Version with markings to show changes made".

IN THE SPECIFICATIONS:

The last sentence in following paragraph [0009]has been deleted as shown:

[0009] Interesting hypothesis has been proposed that sporadic Alzheimer disease might be the brain type of non-insulin dependent diabetes mellitus (Hoyer, S. Is sporadic Alzheimer disease the brain typr of non-insulin dependent diabetes mellitus? A challenging hypothesis. J. Neural Transm. 105, 415-422, 1998). It has been suggested that intracerebroventricular insulin enhances memory in a passive-avoidance task [Park, C. P., Seeley, R. J., Craft, S. and Woods S. C. (2000) Intracerebroventricular insulin enhances memory in a passive avoidance task. Physiol. Behav. 68, 509-514]. Insulin receptor density and tyrosine kinase activity in the sporadic Alzheimer's disease (SDAT) was known to be significantly decreased [Frolich, L., Blum-degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., Muschner, D., Thalheimer, A., Turk, A., Hoyer, S., Zochling, R., Boissl, K. W., Jellinger, K., and Piederer, P. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. J. Neural Transm. 105, 423-438, 1998]. Interestingly, tyrosine phosphorylation of the hippocampal insulin receptor has been shown to play an essential role in spatial memory formation [Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M. J., Alkon, D. L. (1999) Brain insulin receptors and spatial memory. J. Biol. Chem. 274, 34893-34902, 1999]. ~~Taken together, insulin receptor activators could be used for memory enhancement in addition to cholinesterase inhibitors.~~

A NEW paragraph numbered [0010] has been added after paragraph [0009] in the original application as follows:

[0010] Recently, it has been found that ERK (Extracellular signal-Regulated Kinase or MAPK) I and II, which are important downstream signaling mediators of the insulin receptor, are implicated in memory and learning [Thiels, E, Klann, E. Extracellular signal-regulated kinase, synaptic plasticity, and memory. Rev. Neurosci. 12, 327-345, 2001; Sweat J.D. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J. Neurochem. 76, 1-10, 2001]. It has been also demonstrated that rats subjected to avoidance learning showed significant and specific increases in the

activated forms of ERK I and II in the rat hippocampus [Cammarota, M., Bevilaqua, L.R.M., Ardenghi, P., Paratcha, G., de Stein, M.L., Iaqueirdo, I., Medina, J.H. Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning; abolition by NMDA receptor blockade. Mol. Brain Res. 76, 36-46, 2000]. Taken together, insulin receptor and ERK I/II activators could be used for memory enhancement in addition to cholinesterase inhibitors.

Paragraph[0072] has been replaced with the following rewritten paragraph:

[0072] The test was basically performed according to the step-through method described by Jarvik and Kopp [Jarvik, M. E. and Kopp, R. An improved one-trial passive avoidance learning situation. Pschol. Rep. 21, 221-224, 1967]. The Gemini Avoidance System (SD Instruments) was used for this experiments. The apparatus consists of a two-compartment acrylic box with a lightened compartment connected to a darkened one by an automatic guillotine door. Mice were placed in the lighted box for 300 sec. Then, the guillotine door was open. Mice, as soon as they entered the dark compartment, received a punishing electrical shock (0.3 mA, 1 sec). The latency times for entering the dark compartment were measured in the training test and after 24 hr in the retention test. The maximum entry latency allowed in the retention session was 500 sec. Fraction 1, 2 or 4 (10 mg/kg/day, P.O.) was administered once a day for three days and tested for the passive avoidance test.

Paragraph[0078] has been replaced with the following rewritten paragraph:

[0078] Male Sprague Dawley rats were decapitated after 60 min. following the administration of AR extracts and subjected to the isolation of hippocampus on 4C.. Hippocampal homogenates were prepared as described earlier with some modifications [Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M. J., Alkon, D. L., Insulin receptors and spatial memory. J. Biol. Chem. 274, 34893-34902, 1999]. The isolated hippocampus was resuspended with buffer A containing 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, 0.5 mM PMSF, 1 mM Na_3VO_4, 1ug/ml of leupeptin and aprotinin and subjected to homogenization with a Potter-Elvehjem homogenizer. The lysates were then spun at ~~1,000 x g for 5 min~~ 10,000 x g for 20 min and the supernatant were subjected to protein assay and saved at 70°C.

Paragraph[0083] has been replaced with the following rewritten paragraph:

[0083] Equal amount of hippocampal proteins were applied to SDS polyacrylamide gel. Electrotransfer of proteins from the gels to nitrocelluose paper (Schleicher & Schuell) was carried out for 1 hr at 100 V (constant) as described by Towbin et al. [Towbin H., Staehelin, J., Gordon, J. Electric transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Aci. USA 76, 4350-4354, 1979].The filter papers were preincubated for 1 hr at 23 C with PBS containing 0.1% Tween 20 and 3% bovine serum albumin and washed with PBS containing 0.1% Tween 20 three times for 10 min each. The blots were probed with pTyr or pERK antibodies for 1 hr at 23 C. The blots were then incubated with HRP-conjugated anti-rabbit IgG for 30 min and washed with PBS containing Tween 20 five times for 10 min each. The detection of immobilized specific antigens was carried out by ECL (NEN).

Paragraph[0088] has been replaced with the following rewritten paragraph:

[0088] Male SD rats were dosed p.o. with vehicle or fractions of AR extract. The rats were decapitated after ~~90~~ 60 min, brains rapidly removed, hipocampus and corpora striata dissected free, weighed and homogenized as described above. Cholinesterase activity was measured as described by Ellman et al [Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.1961]. Briefly, 3 ml of buffer I (100 mM phosphate, pH 8.0), 0.2 ml of 75 mM acetylthiocholine iodide and 0.1 ml of buffered Ellman's reagent (DTNB 10 mM, NaHCO3 15 mM) were mixed and allowed to incubate for 10 min at 25°C. Then, 20 ml of enzyme sample was added and absorbance was measured at 30 sec intervals. The percent inhibition was calculated by comparison with the enzyme activity of the vehicle control group.

IN THE CLAIMS:

The following claims have been amended:

1. (Amended) A composition containing Asiasari Radix extracts having at least two therapeutically effective agents therein for protecting brain cells against damage caused by

excitatory amino acids and oxidative stresses. ~~and improving memory~~.

11. (Amended) The composition of Claim 1, wherein said the Asiasari Radix extracts are obtained by the following sequential fractionation procedure:

a) extract~~s~~ing ~~of~~ Asiasari Radix ~~obtained according to the method described in the claims 3 are solubalized in a methanol~~: with a lower alcohol mixed with water;

b) ~~water mixed solvent, adjusted~~ing ~~to~~ the pH to 2-4 with acid; ~~and subjected to~~

c) extract~~ion~~ing the solution in step b) with an equal volume of chloroform;

d) isolating a ~~the~~ chloroform insoluble fraction;

e) ~~is then~~ adjusted~~ing to~~ the pH of the solution in step d) to 9-12 with NH4OH; ~~and~~

f) subjected~~ing to~~ the solution in step d) to an extraction with equal volume of a chloroform:methanol mixed solvent; and

~~among these, the chloroform.~~

g) isolating and extracting a methanol insoluble fraction ~~is further extracted~~ from step f) and fractionated~~ing~~ the same with methanol to obtain the Asiasari Radix extracts which are methanol soluble.

12. (Amended) A composition for improving memory containing a chloroform fraction of Asiasari Radix extracts obtained by the following sequential fractionation procedure: subjecting Asiasari Radix ~~is subjected~~ to extraction with a lower alcohol having between 1 carbon atom and 4 carbon atoms ~~such as methanol or ethanol,~~ or organic solvent, ~~such as acetone, chloroform, methylene chloride, ether, or ethylacetate,~~ the resulting Asiasari Radix extract~~s~~ ~~are~~ being solublized in a methanol:water mixed solvent, having a pH adjusted to ~~pH~~ 2-4 with acid and subjected to extraction with equal volume of chloroform to obtain ~~the~~ a chloroform fraction of Asiasari Radix extracts.

14. (Amended) The composition for improving memory of Claim 12, wherein ~~said~~ the composition further contains carriers, excipients and diluent~~s~~ ~~additionally~~.

15. (Amended) The composition for improving memory of Claim 12, wherein said the composition ~~is formulated as~~ administered via a formulation selected from the group consisting of an oral preparation~~s~~ containing powder~~s~~, tablet~~s~~, capsule~~s~~, suspension~~s~~, syrup~~s~~, ~~and~~ aerosol~~s~~, topical agent, ~~external applications~~, suppository~~ies~~ and sterile injection~~s~~.